3

A further aspect of the invention is directed to a method for the identification of a nucleotide sequence encoding a polypeptide comprising any one of the following activities from S. degradans: cellulose depolymerase, or cellulose binding. An S. degradans genomic library can be constructed 5 in *E. coli* and screened for the desired activity. Transformed *E.* coli cells with specific activity are created and isolated.

Further aspects of the invention are directed to utilization of the cellulose degrading substances in food, beer, wine, animal feeds, textile production and laundering, pulp and 10 paper industry, and agricultural industries.

Other aspects, features, and advantages of the invention will become apparent from the following detailed description, which when taken in conjunction with the accompanying figures, which are part of this disclosure, and which illus-15 trate by way of example the principles of this invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A shows the chemical formula of cellulose:

FIG. 1B illustrates the physical structure of cellulose;

FIG. 2A illustrates the degradation of cellulose fibrils;

FIG. 2B shows the chemical representation of cellulose degradation to cellobiose and glucose;

culture supernatants;

FIG. 4 lists the predicted cellulases of S. degradans 2-40 the sequences from FIGS. 4-40 are disclosed as SEC) ID NOs 1-214, respectively in order of appearance, 1—Acronyms, cel=cellulase, ced=cellodextrinase, bql=6-glucosidase, 30 cep=cellobiose/cellodextrin phosphorylase; 2—Protein identified by tandem mass spectrometry in supernatant concentrates. Growth substrates: av=avicel; aq=agarose; al=alginate, cm=CMC, xn=xylan; 3-MW and amino acid count calculated using the protParam (protein parameters) 35 tool at the Expasy website based on the DOE/JGI gene model amino acid sequence translations and includes any predicted signal peptide; 4—Predictions of function and GH, GT and CBM module determination according to CAZy ModO analysis by B. Henrissat, AFMB-CRNS; Daggers (t) indicate 40 lack of a secretion signal sequence; 5—Nonstandard module abbreviations, LPB=lipobox motif, PSL=polyserine linker, EPR=glutamic acid-proline rich region, PLP=phospholipase-like domain, number in parentheses indicates the length of the indicated feature in amino acid 45 residues; 6—Refseq accession number of gene amino acid sequence from the Entrez protein database:

FIG. 5 lists the predicted xylanases, xylosidases and related accessories of M. degradans 2-40;

FIG. 6 lists the predicted pectinases and related accessories 50 of S. degradans 2-40, 1-Acronyms, pel pectate lyase, pes=pectin methylesterase, rql=rhamnogalacturonan lyase; 2—MW and amino acid count calculated using the protParam (protein parameters) tool at the Expasy website based on the DOE/JGI gene model amino acid sequence translations and 55 includes any predicted signal peptide; 3-Predictions of function and GET, GT, PI, CE and CBM module determination according to CAZy ModO analysis by B. Henrissat, AFMB-4—Module abbreviations, CE=carbohydrate esterase, FN3=fibronectin type3-like domain, LPB=lipobox 60 motif, PL=pectate lyase, PSR=polyserine region, EPR=glutamic acid-proline rich region, number in parentheses indicates the length of the indicated feature in amino acid residues; 5—Refseq accession number of gene model amino acid sequence from the Entrez Pubmed database;

FIG. 7 lists the arabinanases and arabinogalactanases of S. degradans 2-40;

FIG. 8 lists the mannanases of S. degradans 2-40;

FIG. 9 lists the laminarinases of S. Degradans 2-40, Superscripts: 1-Acronyms, lam=laminarinase; 2-MW and amino acid count calculated using the protParam (protein parameters) tool at the Expasy website based on the DOE/JGI gene model amino acid sequence translations and includes any predicted signal peptide: 3-Predictions of function and GH. GT, PL, CE and CBM module determination according to CAZy ModO analysis by B. Henrissat, AFMB-CRNS; 4—Module abbreviations: TSP3=thrombospondin type3 repeats, COG3488=thiol-oxidoreductase like domain of unknown function (Interestingly, a similar domain is found in see table 7), PSD=polyserine domain, TMR=predicted transmembrane region. FN3=fibronectin type3like domain, EPR=qlutamic acid-proline rich region, CADG=cadherin-like calcium binding motif, number in parentheses indicates the length of the indicated feature in amino acid residues; 5-Refseq accession number can be used 20 to retrieve the gene model amino acid sequence from the Entrez Pubmed database:

FIG. 10 lists selected carbohydrate-binding module proteins of S. degradans 2-40; and

FIG. 11 lists the recombinant proteins of S. degradans 2-40 FIG. 3 shows SDS-PAGE and Zymogram analysis of 2-40 25 and a comparison of predicted vs. observed molecular weights thereof.

DETAILED DESCRIPTION

Analysis of the genome sequence of S. degradans 2-40 reveals an abundance of genes coding for enzymes that are predicted to degrade plant-derived carbohydrates. To date, 2-40 is the only sequenced marine bacterium with apparently complete cellulase and xylanase systems, as well as a number of other systems containing plant-wall active carbohydrases.

Thus it appears that 2-40 can play a significant role in the marine carbon cycle, functioning as a "super-degrader" that mediates the breakdown of CP from various algal, plantal, and invertebrate sources. The remarkable enzymatic diversity, novel surface features (ES), and the apparent localization of carbohydrases to ES make S. degradans 2-40 an intriguing organism in which to study the cell biology of CP metabolism and surface enzyme attachment.

It has now been discovered that 2-40 has a complete complement of enzymes, suitably positioned, to degrade plant cell walls. This has been accomplished by the following approaches: a) annotation and genomic analysis of 2-40 plant-wall active enzyme systems, b) identification of enzymes and other proteins which contain domains or motifs that may be involved in surface enzyme display, c) the development of testable models based on identified protein motifs, and d) cloning and expression of selected proteins for the production of antibody probes to allow testing of proposed models of surface enzyme display using immunoelectron microscopy.

These efforts have been greatly facilitated by the recent sequencing of the genome of 2-40, allowing a strategy where genes which code for proteins with potential involvement in surface attachment may be identified based on sequence homology with modules or domains known to function in surface attachment and/or adhesion.

Enzymatic and non-enzymatic ORFs with compelling sequence elements are identified using BLAST and other amino acid sequence alignment and analysis tools. Genes of interest can be cloned into E coli, expressed with in-frame polyhistidine affinity tag, fusions and purified by nickel ion